

MINISTRY OF INDUSTRY, COMMERCE, AND CRAFTS
OFFICE FOR DEVELOPMENT OF PRODUCTION
AND COMPETITIVE CAPABILITY

ITALIAN PATENT AND TRADEMARK BUREAU

MANUFACTURING PATENT NO. 01303251

The present patent is being issued for the invention to which the subsequently indicated application pertains

<i>Application Number</i>	<i>Year</i>	<i>Provincial Office --Industry, Commerce, and Crafts</i>	<i>Date for Submission of Application</i>	<i>Classification</i>
002290	1998	Milan	October 26, 1998	C07H019

REGISTRANT: INDUSTRIALE CHIMICA, S.R.L., in Milan

REPRESENTED BY: GEMMA GERVASI

ADDRESS: NOTARBARTOLO & GERVASI, S.P.A.
Corso di Porta Vittoria 9
20122, Milan

TITLE: INDUSTRIAL PROCESS FOR PURIFYING
2', 3'-DIDEHYDRO-3'-DEOXYTHYMIDINE

INVENTORS: MARIACRISTINA FOCHI
ALBERTO SALA

Rome, November 6, 2000

Director of Division XIX:
Giovanna Morelli

1665PTIT

[Duty stamp with illegible
Seal superimposed]To: THE MINISTRY OF INDUSTRY, COMMERCE, AND CRAFTS
ITALIAN PATENT AND TRADEMARK BUREAU, Rome

FORM A

APPLICATION FOR MANUFACTURING PATENT, FILING OF OBSERVATIONS, ADVANCE ACCESSIBILITY FOR THE PUBLIC

A. THE APPLICANT (1):

1) Name: INDUSTRIA CHIMICA, S.r.l. SR
 Address: MILAN Code: 07 4 6 2 4 8 0 1 5 8

2) Name: _____ Code: _____
 Address: _____

B. APPLICANT'S REPRESENTATIVE FOR PATENT AND TRADEMARK OFFICE

Surname, Name: Dr. Gemma Gervasi et al. Fiscal Code: _____
 Name of Firm: Notarbartolo & Gervasi, S.p.A.
 Address: Corsone di Porta Vittoria 9 City: Milan Postal Code: 20122 Province: Milan

C. DESIGNATED LEGAL ADDRESS FOR ADDRESSEE:

Street: _____ No. 11 City: _____ Postal Code: _____ Province: _____

D. TITLE

Proposed Category (Section/Category/Division) C07H Group/Subgroup: _____
Industrial Process for Purifying 2', 3'-didehydro-3'-deoxythymidine

ADVANCE ACCESSIBILITY FOR THE PUBLIC: Yes No

PETITION: DATE: _____ / _____ / _____ Protocol Number: _____

E. DESIGNATED INVENTORS:

Surnames Names	Surnames Names
(1) <u>FOCHI, MARIACRISTINA</u>	(3) _____
(2) <u>SALA, ALBERTO</u>	(4) _____

F. PRIORITY:

Nation or Organization	Type of Priority	Application Date	Filing S/R	Issuing of Observations: Date _____ Protocol No. _____
(1) <u>NONE</u>	_____	_____	_____	_____
(2) _____	_____	_____	_____	_____

G. APPROVED SITE FOR COLLECTING CULTURES OF MICROORGANISMS (Name): _____

H. SPECIAL ANNOTATIONS:

NONE

ACCOMPANYING DOCUMENTATION

No. of Copies

Doc. 1	<u>2</u>	Submitted	No. of Pages <u>14</u> Summary with Principal Drawings, Description, and Claims) (1 copy is obligatory)...	Issuing of Observations: Date _____ Protocol No. _____
Doc. 2	<u>2</u>	Submitted	Item No. <u>01</u> Drawings (Obligatory if cited within description -1 copy).....	_____
Doc. 3	<u>1</u>	RIS	Letter of Appointment, Power of Attorney, or Reference to General Power of Attorney.....	_____
Doc. 4	<u>0</u>	RIS	Designation of Inventor	_____
Doc. 5	<u>0</u>	RIS	Documents concerning Priority, with Italian Translation Comparison Individual Priority.....	[Bob..missing text? C.]
Doc. 6	<u>0</u>	RIS	Authorization or Transfer Agreement.....	_____
Doc. 7	<u>0</u>	RIS	Applicant's Full Name.....	_____

8) Attestations concerning Payments, Total Amount: Three Hundred and Sixty-Five Thousand Lire ObligatoryPREPARED ON October 26, 1998 APPLICANT'S SIGNATURE: GEMMA GERVAZICONTINUATION YES / NO [NO] [Signature]IS A CERTIFIED COPY OF THE PRESENT DOCUMENT BEING REQUESTED? YES / NO [Yes]PROVINCIAL OFFICE--INDUSTRY, COMMERCE, AND CRAFTS: Milan Code: 15FILING REPORT: APPLICATION NUMBER: MI 98/AQ02290 Record AYear: Nineteen hundred and ninety-eight Date: Twenty-sixth Month: OctoberThe previously cited Applicant has submitted the present application to me, accompanied by [00] supplementary pages, for issuing of the previously cited patent.

I. MISCELLANEOUS ANNOTATIONS BY OFFICIAL WHO HAS DRAFTED THIS DOCUMENT: _____

FILED BY
[Signature]

OFFICE SEAL

OFFICIAL WHO HAS DRAFTED DOCUMENT
[Signature] MAURIZIO CORTONESI

1665PTIT

SUMMARY OF INVENTION WITH PRINCIPAL DRAWINGS, DESCRIPTION, AND CLAIMSAPPLICATION NUMBER: M198A002290 Record A
PATENT NUMBER: Filing Date: 1/26/1998
Issuing Date: **B. TITLE:**Industrial Process for Purifying 2', 3'-Didehydro-3'-Deoxythymidine

[Stamped inscription]:Amended Description
(Article 49, Presidential Decree No. 338/1979)
Petition Filed onNovember 11, 1998**L. SUMMARY**

This invention describes a new industrial process for purifying 2', 3'-didehydro-3'-deoxythymidine (d4T; stavudine). This process is characterized by formation of solvates with N, N-dimethylacetamide and by a subsequent desolvation reaction whereby pure stavudine is obtained.

M. DRAWING

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Application for a manufacturing patent entitled:

"Industrial Process for Purifying 2', 3'-Didehydro-3'-Deoxythymidine"

on behalf of: **INDUSTRIALE CHIMICA, S.r.l.**,

whose main office is located in: **MILAN** [stamp] **MI 9 8 A 0 0 2 2 9 0**

Designated inventors: **MARIACRISTINA FOCHI, ALBERTO SALA**

Filed on _____ as Number _____

* * * * *

[Stamped inscription]: **OCTOBER 26, 1998**

FIELD OF THE INVENTION

The present invention pertains to the field of synthesizing antiviral products and especially didehydro-dideoxynucleoside derivatives. The invention describes a process for purifying 2', 3'-didehydro-3'-deoxythymidine (stavudine), which is an antiviral compound which is useful for treating infections caused by the HIV virus.

PRIOR ART

Stavudine (2', 3'-didehydro-3'-deoxythymidine) is a thymidine derivative with antiviral action. This product was recently approved for treating acquired immunodeficiency syndrome (AIDS), especially among patients who are intolerant or refractory to other antiviral therapies (Drugs Future, 1994, 19(10), 925-932; New Products Intros., Drug Persp., 1994, 7(7), 402).

In consideration of the substantial pharmaceutical significance of this molecule, there are many studies whose purpose is to identify an efficient method of preparing it.

Various methods of synthesis have been identified with thymidine as a point of departure, for example:

- Opening the ring within a 3'5'-anhydrous intermediate substance [J.P. Horwitz *et al.*, *J. Org. Chem.*, **32**, 817 (1967)];

- β -elimination of a 3'-selenoxide molecule [J. Vial *et al.*, *Nucleosides & Nucleotides*, **9**, 245 (1990)];
- Reductive β -elimination of an O², 3'-cyclic nucleoside [B.V. Joshi *et al.*, *J. Chem. Soc., Perkin Trans. 1*, 2537 (1992);
- Basic β -elimination by means of a 3'-mesylate [Horwitz *et al.*, *J. AM. Chem. Soc.*, **86**, 1896 (1964)].

A critical point in preparing stavudine consists of purification of the product, which is normally obtained in a crude form upon completion of existing synthesis processes. The purification processes which are ordinarily used, such as chromatography, crystallization, resin treatments, etc., are not highly satisfactory in terms of yields or applicability on an industrial scale. Isolating the product by means of DMSO under intensively basic conditions and at high temperatures results in decomposition of the product (Horwitz *et al.*, *op. cit.*).

In consideration of the previously cited limitations of existing technology, the need for a stavudine purification process which is easily and efficiently applicable on an industrial scale has been recognized.

SUMMARY

The present invention describes a new industrial process for purifying 2', 3'-didehydro-3'-deoxythymidine (d4T; stavudine). The process is characterized by formation of solvates with N,N-dimethylacetamide and by a subsequent desolvation reaction, whereby pure stavudine shall be obtained.

DESCRIPTION OF DRAWINGS:

- A) Figure 1: Synthesizing stavudine**
 - 1) β -thymidine**
 - 2) 5'-O-Benzoylthymidine**
 - 3) 5'-O-Benzoyl-3'-O-methanesulfonylthymidine**
 - 4) 2',3'-Didehydro-3'-deoxythymidine**

DETAILED DESCRIPTION OF THE INVENTION

The present invention describes a new industrial process for purifying crude 2', 3'-didehydro-3'-deoxythymidine (d4T; stavudine).

The process according to the present invention can be used in any situation where obtaining pure stavudine from the respective crude product may be necessary. In particular, it can be applied to any synthesis process resulting in formation of crude stavudine. It can also be used as an alternative for previously described existing procedures for purifying this product. Certain examples of these processes have been cited heretofore during the analysis of prior art.

According to a preferred version, the present process is applicable to synthesis of stavudine as described within the accompanying application which was filed on October 26, 1998 on behalf of the Applicant. This application is included herein for reference. Synthesis of this kind includes treatment of a 5'-O-benzoyl-3'-alkylsulfonyl-thimidine or a 5'-O-benzoyl-3'-O-arylsulfonyl-thymidine, with formation of stavudine in a single stage.

The preferred derivatives for the synthesis in this form are 5'-O-benzoyl-3'-O-mesyl-thymidine or 5'-O-benzoyl-3'-O-tosyl-thymidine.

The base used for this type of synthesis can be a single compound, or it can advantageously consist of a pair of derivatives of a basic nature, preferably (i) sodium or potassium t-butylylate, and (ii) sodium methylate.

The diagram for this synthesis reaction appears within Figure 1.

The purification process to which the present invention pertains is characterized by formation of a solvate with N, N-dimethylacetamide (DMA) and by the subsequent purification-desolvation reaction.

For the purpose of forming a solvate, stavudine is placed within an excessive portion of DMA (from 2 to 10 volumetric units of DMA per gram of stavudine). The solution must undergo stirring at ambient temperature for a period varying between 30 minutes and two hours, and it must then

undergo dilution with a suitable amount of solvent at ambient temperature. Solvents which are suitable for precipitating a solvate from stavudine especially include ethers, ketones, and hydrocarbons. Favorable results have been obtained with use of ethyl ether and isopropyl ether. In order to complete precipitation of the solvate, it may be necessary to allow cooling to 0° C.

The solvate which is obtained in this manner contains from 0.5 to 1 moles of DMA per mole of stavudine, and it unexpectedly offers optimal stability. Indeed, the stability of the solvate is sufficient to allow easy isolation and eventual purification. Moreover, its stability is not excessively high, so that obtaining of pure stavudine by desolvation within appropriate solvents is therefore possible, without being obliged to introduce drastic conditions (high temperatures, long periods of time, etc.) which may be detrimental to stavudine's own purity and stability.

DMA offers the additional advantage of being characterized by low costs and of ensuring high yields of the purified product.

The solvate obtained in this manner can be easily desolvated by treatment with appropriate solvents, so that purified free stavudine can be obtained. It is possible for ketone solvents (acetone, for example), esters (isopropyl acetate, butyl acetate, etc.), and alcohols (for example, isopropanol) to be used, with heating to a temperature between 20° C. and 50° C.

Another form of purification of the solvate consists of dissolving it, which may possibly be facilitated by heating it slightly within DMA, with subsequent reprecipitation by means of suitable solvents such as the previously cited solvents.

When necessary, the previously described purification cycle can be repeated for a second time.

At this point, the present invention shall be described in terms of the following nonrestrictive examples.

Experimental Section

Example 1:

5'-O-Benzoylthymidine

A solution of thymidine (180 g) in pyridine (1,800 ml) must be cooled to 0° C. - 5° C., and it must be gradually treated with benzoyl chloride (121.3 g). Upon completion of addition of the latter substance, the solution is warmed to ambient temperature, and stirring must occur for three days. Most of the pyridine undergoes evaporation under vacuum conditions, and the resulting solution is then poured into water and ice, while vigorous stirring occurs. The suspension must be stirred for two hours, and the solid portion must be filtered and suitably rinsed with water, before being dried under vacuum conditions. The solid which is obtained then undergoes purification in toluene (2,500 ml). The toluene suspension must undergo stirring at 100° C. for 30 minutes, with cooling to 60° C. before being filtered and rinsed inside the filter with heated toluene. If necessary, the purification phase should be repeated another time. 5'-O-benzoyl-thymidine can be recovered with an 84% yield.

Example 2:

5'-O-benzoyl-3'-methanesulfonyl-thymidine

A solution of 5'-O-benzoyl-thymidine (210 g) in pyridine (1,000 ml), which must be cooled to a temperature of less than 10° C., and it must be treated drop by drop with mesyl chloride (83.2 g). Upon completion of addition of the latter substance, the solution is heated to 20° C., and it must undergo stirring for four hours. Evaporation of pyridine is performed under vacuum conditions, and it is dissolved again with water (900 ml) and methylene chloride (1,800 ml). The organic phase then undergoes filtration and cooling at 0° C. - 5° C. Formation of a precipitate occurs, and this precipitate must be filtered and rinsed with cold methylene chloride. After drying, 5'-O-benzoyl-3'-O-methanesulfonyl-thymidine is obtained with a 93% yield.

Example 3:**2', 3'-didehydro-3'-deoxythymidine (Synthesis)**

A solution of 5'-O-benzoyl-3'-O-methanesulfonyl-thymidine (255 g) in N, N-dimethylformamide (1,200 ml) must be treated at a temperature of 20° C. with portions of potassium t-butylate (202 g). Upon completion of addition of the latter substance, stirring is performed at ambient temperature for four hours. The suspension which is obtained is then poured into cold toluene (5,500 ml). Stirring at room temperature is performed for 30 minutes, and the gummy solid which is obtained is redissolved until complete dissolution in water (700 ml) occurs. The toluene phase must then be separated. The aqueous solution is then cooled to a temperature of less than 15° C., and it must be neutralized with concentrated hydrochloric acid before undergoing evaporation under vacuum conditions at approximately 30° C. - 35° C. Traces of water can be removed by treating the remaining product with butanol and by evaporation under vacuum conditions until drying occurs. The remaining product is then extracted with portions of acetone (total of 2,000 ml). The solution must then be treated with carbon, filtered, and evaporated under vacuum conditions, with crude 2', 3'-didehydro-3'-deoxythymidine being obtained.

Example 4:**2', 3'-didehydro-3'-deoxythymidine (synthesis with a pair of bases)**

A solution of 5'-O-benzoyl-3'-O-methanesulfonyl-thymidine (255 g) in N,N-dimethylformamide (900 ml) and tetrahydrofuran (900 ml) is treated at a temperature of 20° C. with portions of potassium t-butylate (169 g). Upon completion of addition of the latter substance, stirring is performed at ambient temperature for one hour, and subsequently, the solution is treated by portions of sodium methylate (25 g). Stirring is continued at ambient temperature for one hour. The suspension which is obtained must then be poured into cold toluene (5,500 ml). Stirring is performed at ambient temperature for 30 minutes, and the gummy solid which is obtained is filtered. It is then added to water (700 ml) until complete dissolution occurs. The toluene phase must be separated. The aqueous solution must be cooled to a temperature of less than 15° C.,

and it must be neutralized with concentrated hydrochloric acid before undergoing vacuum evaporation at approximately 30° C. - 35 ° C. Traces of water are removed by treating the remaining product with butanol and by performing evaporation under vacuum conditions until drying occurs. The remaining product is extracted with acetone according to portions (total of 2,000 ml). The solution is then treated with carbon, filtered, and evaporated under vacuum conditions, until crude 2', 3'-didehydro-3'-deoxythymidine is obtained.

Example 5:

2', 3'-didehydro-3'-deoxythymidine (purification)

Crude 2', 3'-didehydro-3'-deoxythymidine (obtained according to Example 3) is treated at ambient temperature with N, N-dimethylacetamide (DMA, 500 ml) (For obtaining complete dissolution, it is necessary to apply heat for several minutes at 40° C.). The solution is stirred at ambient temperature for 30 minutes, and it is then treated with isopropyl ether (1,100 ml). The solid substance which is obtained must be stirred for two hours while it is cool, and it must be filtered.

'H-NMIR analysis of a sample after drying reveals formation of a solvate by 2', 3'-didehydro-3'-deoxythymidine and DMA with a molar ratio of 1/0.75:

'H-NMR (DMSO, ppm): 11.27 (s, 1H), 7.62 (s, 1H), 6.37 (d, 1H), 5.89 (d, 1H), 4.97 (m, 1H), 4.76 (m, 1H), 3.59 (m, 2H), 2.93 (s, 2.3H), 2.76 (s, 2.3H), 1.94 (s, 2.3H), 1.71 (s, 3H).

The solid substance is then suspended in DMA (170 ml) and is treated with isopropyl ether (250 ml) at ambient temperature, with stirring for one hour. After filtration, it must be dissolved in isopropanol (700 ml) under heat, and it must be treated with carbon and filtered. Isopropanol must then be concentrated under vacuum conditions (removal of approximately 400 ml of the solvent), and the solution must be cooled. After filtration and drying, 2',3'-didehydro-3'-deoxythymidine can be isolated with a yield of more than 40% (computed by means of crude 5'-O-benzoyl-3'-O-methanesulfonyl-thymidine) and with an HPLC purity of 99.1%.

The structure of this substance can be confirmed by means of $^1\text{H-NMR}$ analyses (DMSO, ppm): 11.27 (s, 1H), 7.62 (s, 1H), 6.80 (m, 1H), 6.37 (d, 1H), 5.89 (d, 1H), 4.97 (m, 1H), 4.76 (m, 1H), 3.59 (m, 2H), 1.71 (s, 3H).

Example 6:

2', 3'-didehydro-3'-deoxythymidine (purification)

Crude 2', 3'-didehydro-3'-deoxythymidine (50% of the amount obtained within Example 4) is treated at ambient temperature with N,N-dimethylacetamide (DMA, 250 ml) (For obtaining complete dissolution, it is necessary to apply heat for several minutes at 40° C.). The solution is stirred at ambient temperature for 30 minutes, and it is then treated with isopropyl ether (550 ml). The solid substance which is obtained must be stirred for two hours while it is cool, and it must be filtered.

Analysis of a sample with $^1\text{H-NMR}$ after drying reveals formation of a solvate by 2', 3'-didehydro-3'-deoxythymidine and DMA with a molar ratio of 1/0.75 (Data consistent with the data provided within Example 5).

The solid substance is then dissolved in DMA (85 ml) and is heated to 40° C., and it is treated with isopropyl ether (125 ml) at ambient temperature, while being stirred for one hour. Isopropanol must then be concentrated under vacuum conditions (removal of approximately 200 ml of the solvent), and the solution must be cooled. After filtration and drying, 2',3'-didehydro-3'-deoxythymidine can be isolated with a yield of more than 50% (computed by means of crude 5'-O-benzoyl-3'-O-methanesulfonyl-thymidine) and with an HPLC purity of 99.0%.

$^1\text{H-NMR}$ analysis has confirmed the structure of stavudine (data consistent with that which was provided in Example 5).

Example 7**2', 3'-didehydro-3'-deoxythymidine (stavudine); purification with:**

(a) N,N-diethylformamide

Crude 2', 3'-didehydro-3'-deoxythymidine (1 g) is treated at ambient temperature with N, N-diethylformamide (2ml) (In order to obtain complete dissolution, heating for several minutes at approximately 50° C. is necessary). The solution must then be stirred at ambient temperature for 30 minutes, and it must subsequently be treated with isopropyl ether (5 ml). An oil which is gradually transformed into a gummy solid is gradually separated at ambient temperature. After filtration and drying, 0.45 g of partially purified stavudine (yellow solid) (TLC eluent: methylene chloride- methanol-9-1) is obtained. Analysis with 'H-NMR demonstrates the presence of mere traces of N, N-diethylformamide, and isolation of a solvate form can therefore be excluded.

(b) N, N-diethylacetamide

Crude 2', 3'-didehydro-3'-deoxythymidine (1 g) is treated at ambient temperature with N, N-diethylformamide (2ml) (In order to obtain complete dissolution, heating for several minutes at approximately 50° C. is necessary). The solution must then be stirred at ambient temperature for 30 minutes, and it must subsequently be treated with isopropyl ether (5 ml). An oil which is gradually transformed into a gummy solid is gradually separated at ambient temperature. After filtration and drying, 0.60 g of partially purified stavudine (yellow solid) (TLC eluent: methylene chloride- methanol-9-1) is obtained. Analysis with 'H-NMR demonstrates the presence of mere traces of N, N-diethylformamide, and isolation of a solvate form can therefore be excluded.

c) N-methylpropionamide:

Crude 2', 3'-didehydro-3'-deoxythymidine (1 g) is treated at ambient temperature with N, N-methylpropionamide (2ml) (In order to obtain complete dissolution, heating for several minutes at approximately 50° C. is necessary). The solution must then be stirred at ambient temperature for 30 minutes, and it must subsequently be treated with isopropyl ether (5 ml). A dense oil which is

gradually transformed into a gummy solid is gradually separated at ambient temperature. After filtration and drying, 0.65 g of partially purified stavudine (whitish solid) (TLC eluent: methylene chloride- methanol-9-1) is obtained. Analysis with $^1\text{H-NMR}$ demonstrates the presence of mere traces of N, N-methylpropionamide and isolation of a solvate form can therefore be excluded.

Example 7 demonstrates that oils or gummy solids which are obtained by the reaction between stavudine and primary or secondary amines other than DMA only contain modest proportions of the solvent, thereby confirming the fact that a solvate does not form or is at least unstable.

The stavudine which was obtained in Example 7 with amides other than DMA possessed unacceptable quality in terms of purity and crystalline properties.

CLAIMS

1. A process for purifying 2', 3'-didehydro-3'-deoxythymidine (stavudine) characterized by the fact that said process includes solvation of stavudine by a reaction with N, N-dimethylacetamide (DMA) and subsequent desolvation of the solvate which is obtained.
2. A process according to Claim Number 1, where the solvate which is obtained contains (DMA) and stavudine in molar ratios situated between 0.5: 1 and 1:1.
3. A process according to Claim Number 1, where desolvation is accomplished by treating solvates at temperatures situated between 20° C. and 50° C. within solvents selected among amides, ethers, ketones, esters, and alcohols.
4. A process according to Claim Number 3, where the solvent used for desolvation is selected among butyl acetate, isopropanol, and acetone.
5. A process according to Claim Number 1, where the stavudine which is to be purified has been obtained by means of a process which includes treatment of 5'-O-benzoyl-3'-O-alkylsulfonylthymidine or 5'-O-benzoyl-3'-O-alkylsulfonylthymidine in the presence of a base, with stavudine being formed during a single stage.
6. An N, N-dimethylacetamide (DMA)/stavudine solvate with molar ratios situated between 0.5:1 and 1:1.
7. A solvate according to Claim Number 6, where the molar ratio between N,N-dimethylacetamide (DMA) and stavudine is equivalent to 0.75:1.

(GER/pd)

[initials]

Milan, October 26, 1998

On behalf of *INDUSTRIALE CHIMICA, S.r.l.*

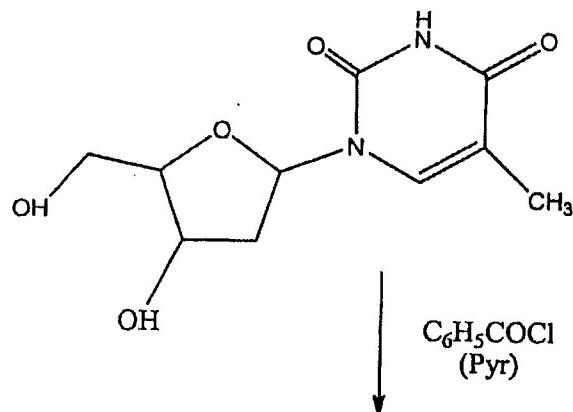
Agent:

[Signature]
Dr. Gemma Gervasi

NOTARBARTOLO & GERVASI, S.p.A.

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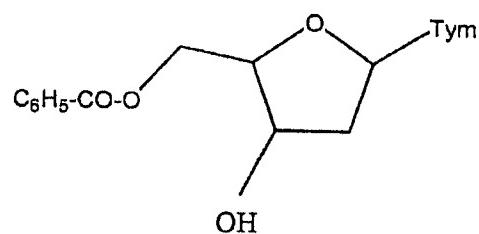
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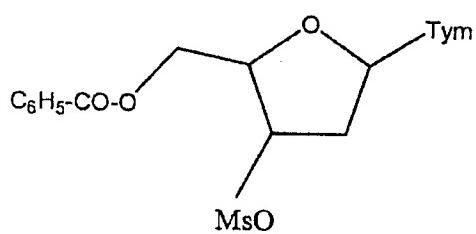
Key:
 { (Pyridine)}

MI98A002290



(2)

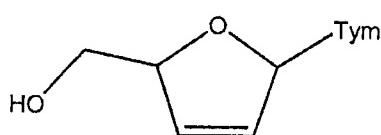
Key:
 { (Pyridine)}



(3)

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MeONa/ButOK
 (DMF-THF)



(4)

[initials]

PETITION

1665PTIT

Protocol No.: _____

[Seal]

MINISTRY OF INDUSTRY, COMMERCE, AND CRAFTS

Office for Development of Production and Competitive Capability [Stamped inscription]:

ITALIAN PATENT AND TRADEMARK BUREAU

Patent MI. V.

COPY OF STATEMENT CONCERNING FILING OF PETITIONS AND DOCUMENTS [002640]

In the year **1998**, on the **eleventh** day of the month of **November**,

the company known as **INDUSTRIA CHIMICA, s.r.l.**,

whose main office is located in **Milan**,

whose agents are: **Dr. Diego Pallini (Registration Number 484)**,

and whose designated address for legal purposes is **Corso di Porta Vittoria 9**,

at the offices of **Notarbartolo & Gervasi, S.p.A.** in Milan,

- subsequent to an application for a **PATENT**, which was filed in **Milan**, on **October 26, 1998**, according to

Protocol Number **MI98A002290**,

filed the subsequently indicated documents with this Office:

1. **PETITION FOR CORRECTION AND INCLUSION**
2. **PAGE 4, ANNOTATION**
3. **PAGE 4, EX NOVO**
- 4.
- 5.

[Seal]: Ministry of Industry, Commerce, and Crafts
Province of Milan Office for Industry, Commerce,
and Crafts, Patent Bureau

APPLICANT:

[Signature]

ISSUING OFFICIAL:

[Signature]

Giuseppe Rescali

For copy identical to the original.

"It is specified that, for an application of this kind and for accompanying duties for stamps shall be waived in accordance with Bulletin Number 163/83 issued by the Patent Bureau, and subsequent amendments thereto, subject to possible additional duties which may be requested by said Bureau within the context of approval."

On behalf of the Director:

(Pier Daniele Melegari)

[Illegible signature]

Dr. M. Colosimo

MINISTRY OF INDUSTRY, COMMERCE, AND CRAFTS

Italian Patent and Trademark Bureau

Rome

Application for a Manufacturing Patent whose title is:

[Duty stamp with
seal superimposed]

"Industrial Process for Purifying 2', 3'-Didehydro-3'-Deoxythymidine"

on behalf of: **INDUSTRIALE CHIMICA, S.r.l.**

whose main office is located in **MILAN**.

Filed on **OCTOBER 26, 1998**, with the number **MI98A002290**.

[Stamped inscription]:
PATENT MI-V.
002640

* * * * *

PETITION FOR CORRECTION AND INCLUSION

On the basis of Article 49 of Presidential Decree Number 338, which was issued on June 22, 1979, the undersigned company, INDUSTRIALE CHIMICA, S.r.l., whose main office is located in Milan, is requesting through its Agent, Dr. Diego Pallini (Registration Number 484), who is affiliated with NOTARBARTOLO & GERVASI, S.p.A., of Corso di Porta Vittoria 9, in Milan, which has been designated as a legal address for all legal purposes, that permission should be granted for providing the following correction, as indicated hereinafter.

NOTE 1, Page 4, Line 21, after the expression "likewise pending," insertion of "Number MI98A002289."

Milan, November 11, 1998.

[stamped inscription]

Commerce, and Crafts.
November 11, 1998
Milan

On behalf of **INDUSTRIALE CHIMICA, S.r.l.**
Provincial Office for Industry,

Agent:

[Signature]

Dr. Diego Pallini

[Seal] Ministry of Industry, Commerce, and Crafts

NOTARBARTOLO & GERVASI, S.p.A.

Province of Milan Office for Industry,
Commerce, and Crafts, Patent Bureau

... obtaining pure stavudine.

DESCRIPTION OF DRAWINGS

A) Figure 1: Synthesizing stavudine

- 1) β -thymidine
- 2) 5'-O-Benzoylthymidine
- 3) 5'-O-Benzoyl-3'-O-methanesulfonylthymidine
- 4) 2', 3'-Didehydro-3'-deoxythymidine

[Stamped inscription]:

PATENT MI-V.
002640

DETAILED DESCRIPTION OF THE INVENTION

The present invention describes a new industrial process for purifying crude 2', 3'-didehydro-3'-deoxythymidine (d4T; stavudine).

The process to which the present invention pertains is usable under any circumstances where obtaining pure stavudine from the respective crude product is intended. In particular, this method may be applied to any synthesis process which may result in formation of crude stavudine; it can also be used as an alternative to existing purification processes which have been described for this product. Some examples of these processes have been cited heretofore in analyzing prior art.

According to a preferred version, the present process is applicable to synthesis of stavudine, as described within concurrently pending Application Number MI98A002289, which was filed on behalf of the Applicant on October 26, 1998, and is included herein for reference. This form of synthesis includes treatment of 5'-O-benzoyl-3'-O-alkylsulfonylthymidine, or 5'-O-benzoyl-3'-O-arylsulfonylthymidine in the presence of a base, with formation of stavudine occurring in a single stage.

[Stamped inscription]: November 11, 1998. Milan.

[Seal]: Ministry of Industry, Commerce, and Crafts

Province of Milan Office for Industry, Commerce,
and Crafts, Patent Bureau